
Major Lakes Phytoplankton Study: Comparison of Composite Sampling Techniques

September 2004



King County

Department of Natural Resources and Parks
Water and Land Resources Division

Science Section

King Street Center, KSC-NR-0600
201 South Jackson Street, Suite 600
Seattle, WA 98104
206-296-6519 TTY Relay: 711
dnr.metrokc.gov/wlr

Alternate Formats Available

206-296-7380 TTY Relay: 711

Major Lakes Phytoplankton Study: Comparison of Composite Sampling Techniques

Submitted by:

Curtis DeGasperi
King County Water and Land Resources Division
Department of Natural Resources and Parks



King County

Department of Natural Resources and Parks

Water and Land Resources Division

201 S Jackson St. Ste 600

Seattle, WA 98104

(206) 296-6519

Acknowledgements

The author acknowledges the contributions of time and resources from the King County Environmental Laboratory (KCEL). In particular, I thank Katherine Bourbonais for managing the field and laboratory aspects of this study. Jeff Droker and David Robinson from KCEL also deserve a great deal of credit for implementing the field study design and coordinating the field study implementation with Katherine.

Citation

King County. 2004. Major Lakes Phytoplankton Study – Comparison of Composite Sampling Techniques. Prepared by Curtis DeGasperi, Water and Land Resources Division. Seattle, Washington.

Table of Contents

Executive Summary	v
1.0. Introduction.....	1
1.1 Study Area	1
1.2 Project Background.....	2
1.3 Goals and Objectives	2
1.4 Historical Data Review	2
2.0. Methods.....	5
2.1 Study Approach	5
2.2 Field Study Plan.....	5
2.3 Laboratory Analysis.....	6
3.0. Results.....	7
3.1 Individual Paired Comparisons.....	7
3.2 Seasonal Comparisons	8
4.0. Discussion.....	9
5.0. Conclusions and Recommendations	17
6.0. References.....	19

Figures

Figure 1 Historical chlorophyll <i>a</i> data for Lake Washington Station 0852.....	3
Figure 2 Routine Major Lakes monitoring stations, RUSS buoy locations, and Hydrodynamic Study locations where quantitative phytoplankton and chlorophyll <i>a</i> data are collected.....	4
Figure 3 Chlorophyll <i>a</i> data for Lake Washington Station 0852 during the paired composite sampling study. Monthly surface grab data also shown for comparison.....	7

Figure 4	Discrete grab profiles of chlorophyll <i>a</i> for Station 0852 in 2002, 2003, and 2004.....	10
Figure 5	Comparison of discrete grab profiles of chlorophyll <i>a</i> for Station 0852 with significantly different discrete and integrated composite results.....	11
Figure 6	South Lake Washington (WASHS) RUSS chlorophyll profiles, May 5 and 19, 2003.	12
Figure 7	South Lake Washington (WASHS) RUSS chlorophyll color contour plot, 2001-2004.	13
Figure 8	SCAMP fluorescence color contour plots (in units of voltage) for Lake Washington and Lake Sammamish, 2003. Black lines indicate date-centered temperature profiles associated with the interpolated fluorescence profiles.....	14

Tables

Table 1.	Sample Containers, Preservation, Holding Times and MDLs.....	6
Table 2.	Results from the paired composite sampling for chlorophyll <i>a</i> in µg/L.....	8
Table 3.	Seasonally aggregated results from the paired composite sampling for chlorophyll <i>a</i> in µg/L.....	8

Appendices

Figure A1.	Box plot comparing paired composite sampling results for May 5, 2003.....	2
Figure A2.	Box plot comparing paired composite sampling results for May 19, 2003.....	2
Figure A3.	Box plot comparing paired composite sampling results for July 7, 2003.	3
Figure A4.	Box plot comparing paired composite sampling results for July 21, 2003.	3
Figure A5.	Box plot comparing paired composite sampling results for August 4, 2003.	4
Figure A6.	Box plot comparing paired composite sampling results for August 18, 2003.	4
Figure A7.	Box plot comparing paired composite sampling results for October 7, 2003.	5
Figure A8.	Box plot comparing paired composite sampling results for October 21, 2003.	5
Figure A10.	Box plot comparing paired composite sampling results for April 20, 2004.....	6

Figure A11. Box plot comparing paired composite sampling results for May 4, 2004.....	7
Figure A12. Box plot comparing paired composite sampling results for May 18, 2004.....	7
Figure A13. Box plot comparing paired composite sampling results for Spring (May) 2003.	8
Figure A14. Box plot comparing paired composite sampling results for Summer (Jul-Aug) 2003.	8
Figure A15. Box plot comparing paired composite sampling results for Fall (Oct) 2003.	9
Figure A16. Box plot comparing paired composite sampling results for Spring (Apr-May) 2004.	9

EXECUTIVE SUMMARY

The Major Lakes Phytoplankton Study was initiated in March 2003. This study involved the collection of integrated composite samples of surface water for phytoplankton species identification, enumeration, and estimation of species-specific phytoplankton biovolume. In addition to the phytoplankton work, a change in the surface water compositing scheme for chlorophyll *a* and phytoplankton taxonomic work was proposed. The previous technique mixed equal parts of samples collected from 1 m below the water surface and at the measured Secchi depth (hereafter referred to as a “discrete composite”). The technique proposed for phytoplankton sampling and for future routine composite sampling for chlorophyll *a* involved the use of a 10-m long 1.6-cm diameter (ID) tube suspended from the surface. The tube is plugged at the surface and at the submerged end by a check valve and retrieved. The submerged tube collects a vertically integrated sample of the surface 10 m of the lake. The sample is decanted into a stainless steel bowl and homogenized before sub-sampling for chlorophyll *a* and phytoplankton enumeration. This sample type will hereafter be described as an “integrated composite”. Paired sampling at the Lake Washington Station 0852 off Madison Park was proposed to evaluate potential differences between the two compositing techniques.

The study design was based on detecting a minimum difference of 1 µg/L of chlorophyll *a* between a group of 20 samples from each sampling technique in a given season. It turned out that the field sampling and analytical variation was small enough to allow statistically significant differences of a few tenths of a µg/L to be detected. Observed differences between the two methods were not consistent, but they could frequently be explained by the apparent vertical variation in phytoplankton chlorophyll concentrations (inferred from the discrete grab profile data or available chlorophyll fluorescence profiles) over the depth interval sampled by each method. The discrete composite sampling interval was typically less than 10 m (i.e., maximum Secchi depth on paired sampling dates was 7.8 m reported in August 2003).

A total of 12 paired sampling events took place between May 2003 and May 2004. Statistically significant differences were detected between integrated vs. discrete composite chlorophyll *a* results in 6 of the 12 sampling events with significant differences ranging from -0.4 to 1.2 µg/L. When the data are grouped into seasons (May 2003, Jul-Aug 2003, Oct 2003, Apr-May 2004), statistically significant differences were only detected in the Jul-Aug 2003 and Oct 2003 periods with differences of 0.4 and -0.2 µg/L, respectively. Although the observed differences between the two methods were often statistically significant, it is questionable if these small differences would significantly affect our ability to detect long term trends in seasonally averaged chlorophyll *a* concentrations in these lakes. This report does not attempt to address this second question. Instead, it is recommended that we re-establish the use of the discrete compositing technique for chlorophyll *a* at selected mid-lake locations (Lake Sammamish: 0611 and 0612; Lake Washington: 0826, 0852, and 0890; Lake Union: A522). This should allow King County to continue the long-term collection of discrete composite chlorophyll *a* data for trend analysis.

As a result of this study and evaluation of discrete chlorophyll *a* profiles at 0852 and available high frequency fluorescence profiling data, additional changes in the methods and frequency of sampling for phytoplankton biomass are proposed for incorporation into the routine monitoring program. Proposed changes include sampling more frequently at a reduced number of stations

and working more cooperatively with University of Washington scientists that are involved in research on Lake Washington and Lake Sammamish. It is believed that a more cooperative working relationship will maximize the use of available resources and provide the best hope of continuing to improve our understanding of how these lakes will respond to environmental change (e.g, population growth and climate change).

1.0. INTRODUCTION

Three major lakes (Lakes Sammamish, Washington, and Union) in King County have been the focus of long-term limnological investigations for several decades. King County Department of Natural Resources and Parks (KCDNRP) is currently developing hydrodynamic and water quality models of these lakes as part of the Sammamish-Washington Analysis and Modeling Program (SWAMP). One goal of this modeling effort is the simulation of multiple algal groups. These groups would represent an aggregation of algal species that have similar environmental requirements/constraints and/or associated management concerns. The groups of algae that will likely be represented initially in the models are diatoms (Bacillariophyceae), green algae (Chlorophyta), and cyanobacteria (Cyanophyta).

The current lakes sampling program provides only qualitative information regarding the presence and relative abundance of individual algal taxa. In order to evaluate the model-predictions of the relative abundance of the representative algal groups, more quantitative phytoplankton data are needed. Specifically, data on the abundance and biovolume of individual algal taxa identified in representative samples collected over time are needed to make quantitative comparisons between the models and field observations.

The Major Lakes Phytoplankton Study was initiated in March 2003. This study involves the collection of integrated composite samples of surface water for phytoplankton species identification, enumeration, and estimation of species-specific phytoplankton biomass. The phytoplankton results will be summarized as part of the lake water quality modeling reports. In addition to the phytoplankton work, a change in the surface water compositing scheme for chlorophyll *a* and phytoplankton taxonomy was proposed. The results of a study comparing the original technique to the proposed composite sampling technique for chlorophyll *a* is the subject of this report.

The previous compositing technique mixed equal parts of samples collected from 1 m below the water surface and at the measured Secchi depth (hereafter referred to as a “discrete composite”). The technique proposed for quantitative phytoplankton sampling and for future routine composite sampling for chlorophyll *a* involves the use of a 10-m long, weighted 1.6-cm diameter (ID) tube suspended from the surface. The tube is plugged at the surface and at the submerged end by a check valve and retrieved. The tube contains a vertically integrated sample of the top 10 m of the lake. The sample is decanted into a stainless steel bowl and homogenized before sub-sampling for chlorophyll *a* and phytoplankton enumeration. This sample type will hereafter be described as an “integrated composite”.

1.1 Study Area

The Major Lakes Phytoplankton Study includes Lake Sammamish, Lake Washington, and Lake Union (Figure 1). The paired comparison study of chlorophyll *a* sampling techniques was conducted at Station 0852 in Lake Washington; the routine monitoring location with the longest sampling record and the only station where discrete profile grab sampling for chlorophyll *a* is conducted on a routine (monthly) basis.

1.2 Project Background

The U.S. Army Corps of Engineers, Engineer Research and Development Center (ACOE-ERDC) has developed a 3-dimensional water quality model (CE-QUAL-ICM) of Lake Washington for KCDNRP. Dispersion and advection of water quality constituents in the water quality model is based on output from a hydrodynamic model of the lake [Curvilinear Hydrodynamics in Three Dimensions (CH3D)]. The water quality model contains at least three state variables for phytoplankton that can be used to represent diatoms, green algae, and cyanobacteria.

In order to compare the spatial and temporal model-predictions of group-specific algal biomass as represented by chlorophyll *a* (the most common surrogate for phytoplankton biomass), measurements of species-specific biomass are needed. Species-specific estimates can then be aggregated to a level that is comparable to that used in the model. By combining group level biovolume data with observed chlorophyll *a* data, group-specific chlorophyll *a* estimates can be derived for model calibration purposes. Ideally, phytoplankton enumeration, biovolume, and chlorophyll *a* measurements would be made on the same sample. It was felt that an integrated composite sample would be less variable and more representative of the surface mixed layer algal population and chlorophyll *a* content than the discrete composite samples. Therefore, it was proposed to implement an integrated compositing method for this project and discontinue the discrete compositing method. Before making a decision to discontinue the discrete compositing method, it was decided that a paired comparison study would be conducted to determine how different the chlorophyll *a* results would be between the two methods.

1.3 Goals and Objectives

The overall goal of this study is the collection of data that will facilitate the development and calibration of lake water-quality models that simulate multiple groups of phytoplankton. An additional goal is to evaluate differences in the reported concentrations of chlorophyll *a* between the original discrete compositing method and the proposed integrated composite sampling technique. The additional goal is the focus of this report.

1.4 Historical Data Review

Presently, KCDNRP collects discrete composite samples for chlorophyll *a* analysis at each Major Lake (Major Lakes are lakes Sammamish, Washington and Union) station on a monthly basis from October through March and twice monthly April through September. At the deeper open water stations, sampling consists of the collection of one (composite) sample at each lake station by compositing discrete samples from 1 m below the surface and at the Secchi depth – the “discrete composite” sample. At some of the shallower nearshore stations where Secchi depths can be greater than the station depth, the deeper grab is collected approximately 1 m above the bottom or just above submerged vegetation. The discrete compositing technique was implemented in March 1994. Discrete grab samples are also collected monthly at Station 0852 in Lake Washington. During summer, the discrete grab sample depths at 0852 are approximately 1, 5, 10, 15, 20, 25, 40, and 60 m. Discrete grab profiling at station 0852 was initiated in April 1993 when this station was first established. Figure 1 presents the historical surface discrete grab

and discrete composite chlorophyll *a* record at Station 0852. Qualitatively, the surface grab data are quite similar to the discrete composite data, with the exception of peak Spring chlorophyll *a* concentrations that were missed in 1997, 1998, 1999, and 2001 by the less frequent discrete grab sampling.

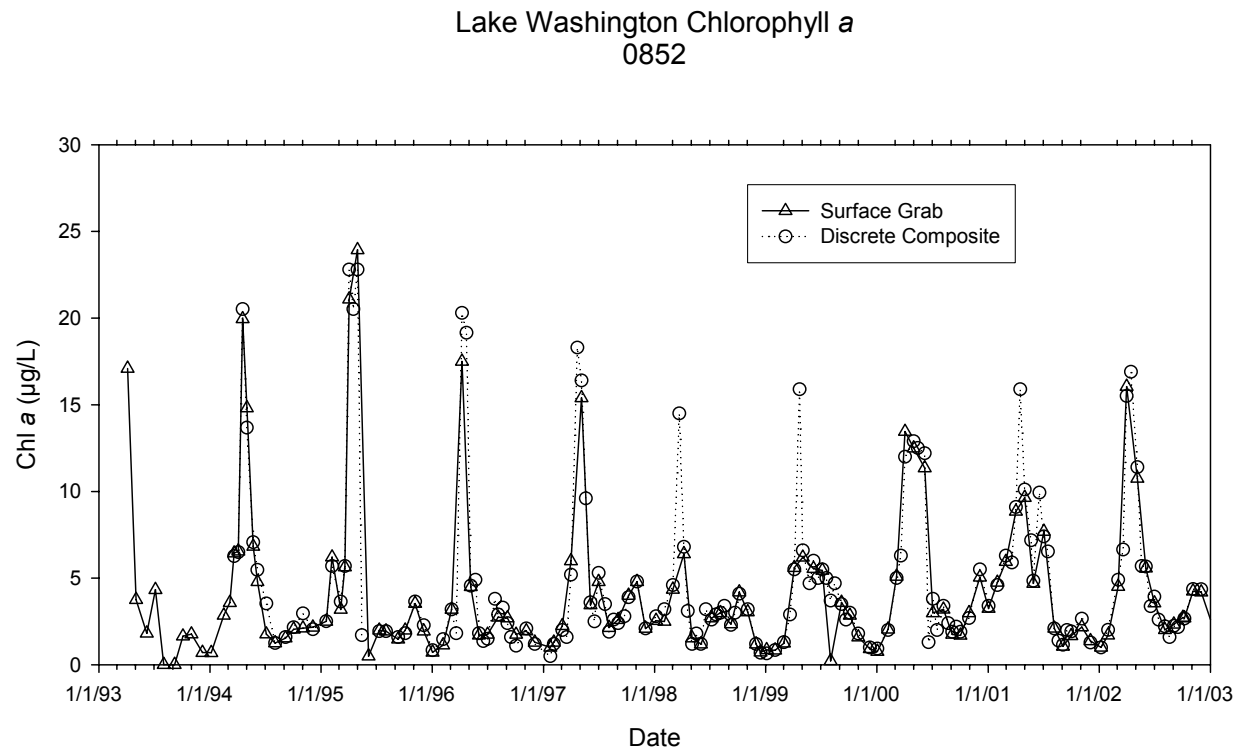


Figure 1 Historical chlorophyll *a* data for Lake Washington Station 0852.

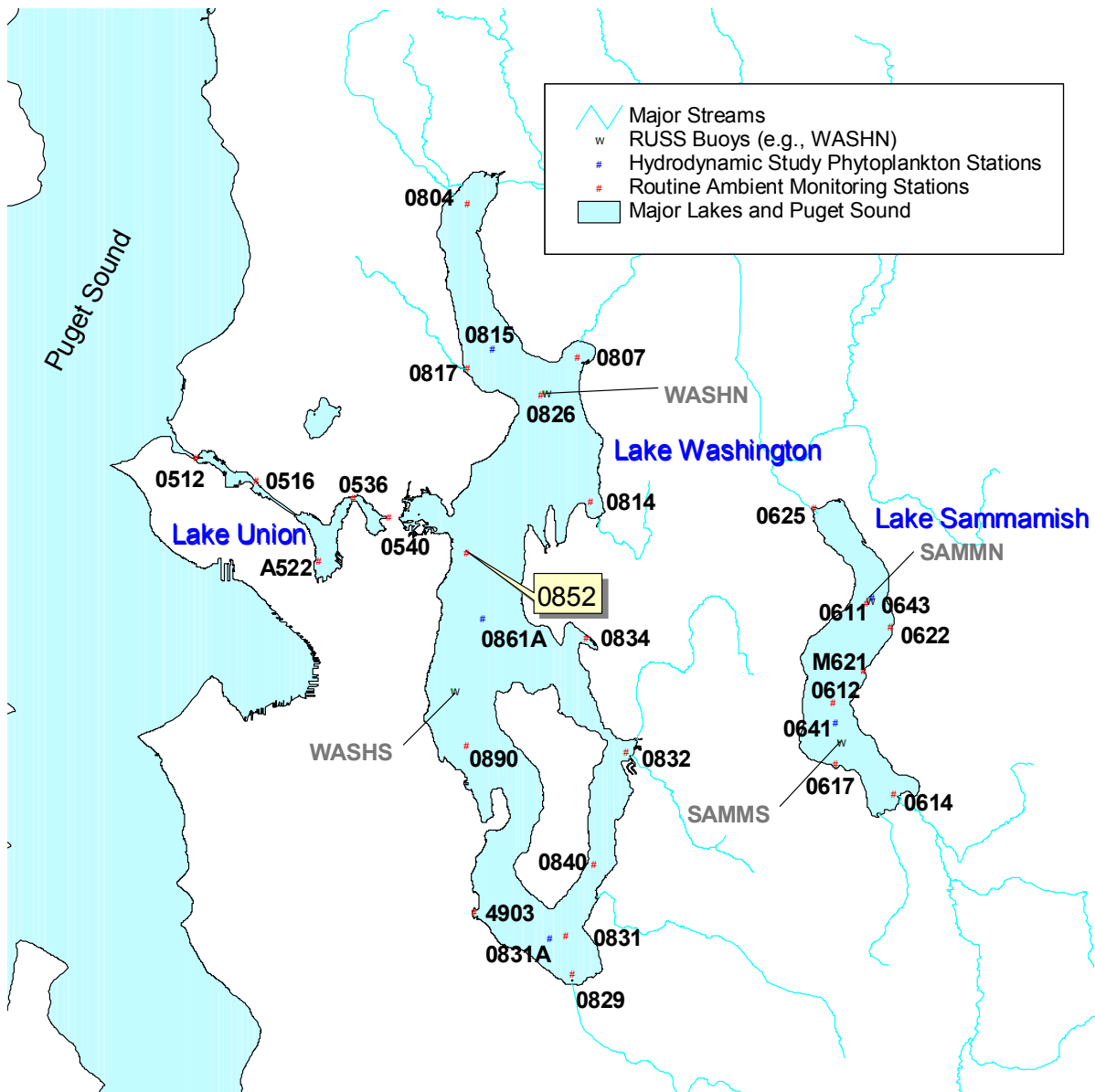


Figure 2 Routine Major Lakes monitoring stations, RUSS buoy locations, and Hydrodynamic Study locations where quantitative phytoplankton and chlorophyll a data are collected.

2.0. METHODS

The study methods and sampling design are described in detail in the study's Sampling and Analysis Plans (King County 2003, 2004). A brief summary of the methods relevant to the paired chlorophyll *a* composite study are provided below.

2.1 Study Approach

The study approach was designed to determine if there is a significant difference in the chlorophyll *a* results obtained using the discrete vs. the integrated composite method. Given estimates of the expected sampling variance, an acceptable difference between the two methods, and the desired probabilities for Type I and Type II errors (alpha and beta, respectively), then one can estimate how many samples should be collected to test the null hypothesis. The null hypothesis (H_0) is: The means of the two sample sets are equal (i.e., $\mu = \mu_0$).

This type of calculation is typically referred to as a power analysis – one minus beta (1-beta) being the estimate of statistical power of the sampling design. Power is the confidence that a Type II error will not occur (i.e., the probability of correctly rejecting a false H_0).

Because it was anticipated that different levels of variance would be expected in particular phytoplankton growing seasons, the design included sampling stratified into spring, summer, and fall periods.

2.2 Field Study Plan

Both current and proposed composite sampling methodologies for chlorophyll *a*/pheophytin were compared by repeated paired sampling at Station 0852 during spring, summer, and fall periods. A total of 20 paired samples (40 samples total for chlorophyll *a*/pheophytin analysis) were collected during each season. The spring 2003 season paired sampling was to consist of 5 pairs of samples (10 samples total) collected at Lake Washington Station 0852 during each of the four bi-weekly sampling events in April and May. However, paired sampling did not begin until May 2003. Therefore, the paired comparison study was extended to include spring 2004 in which paired sampling was conducted during each of the four bi-weekly sampling events in April and May 2003. The summer paired sampling consisted of 5 pairs of samples collected at Station 0852 during each of the four bi-weekly sampling events during July and August. The fall paired sampling consisted of 10 pairs of samples collected at Station 0852 during the two bi-weekly sampling events in October 2003. Note the current compositing scheme conducted at the other routine Major Lakes monitoring stations was modified to match the phytoplankton enumeration and biovolume compositing method (integrated composite sample)¹ described here.

¹ At stations where the total depth is equal to or less than 10 m, the integrated sample will be representative of the water column from the surface to approximately 1 m above submerged vegetation or the bottom.

As noted, chlorophyll *a*/pheophytin sampling at all of the routine monitoring locations was based on a composite of samples collected 1 m below the surface and at the Secchi depth, with one exception. Additional discrete samples have been and will continue to be collected at all depths corresponding to nutrient sampling at Station 0852.

2.3 Laboratory Analysis

Samples collected for analysis of chlorophyll *a* and pheophytin were delivered to the King County Environmental Laboratory (KCEL). Table 1 lists the appropriate containers, preservative, holding times and laboratory method detection limit (MDL) requirements.

Table 1. Sample Containers, Preservation, Holding Times and MDLs.

<u>Analysis</u>	<u>Container</u>	<u>Preservative</u>	<u>Holding Time</u>	<u>MDL</u>
Chlorophyll <i>a</i> ; <u>EPA 446.0</u>	1-L amber plastic, HDPE	4 °C	1 day for filtration 28 days for analysis	0.50 µg/L
Pheophytin <i>a</i> ; <u>EPA 446.0</u>	1-L amber plastic, HDPE	4 °C	1 day for filtration 28 days for analysis	1.0 µg/L

HDPE – High Density Polyethylene

3.0. RESULTS

Unfortunately, the proposed initial spring 2003 sampling period had passed before paired sampling was initiated. Instead of the original set of 5 pairs of samples over April and May, 10 paired sets of samples were collected on May 5th and 19th. The spring bloom peak had already passed before paired sampling was initiated (Figure 3). Paired samples were collected as planned in July, August, and October 2003 and additional paired comparisons were conducted in April and May 2004 resulting in a total of 12 paired sampling events. Routine sampling using the integrated composite method was also conducted beginning in 2003 and routine discrete sampling was re-initiated in 2004 (see below) allowing for additional single sample comparisons between the two sampling methods at Station 0852 beginning in 2004 (see Figure 3).

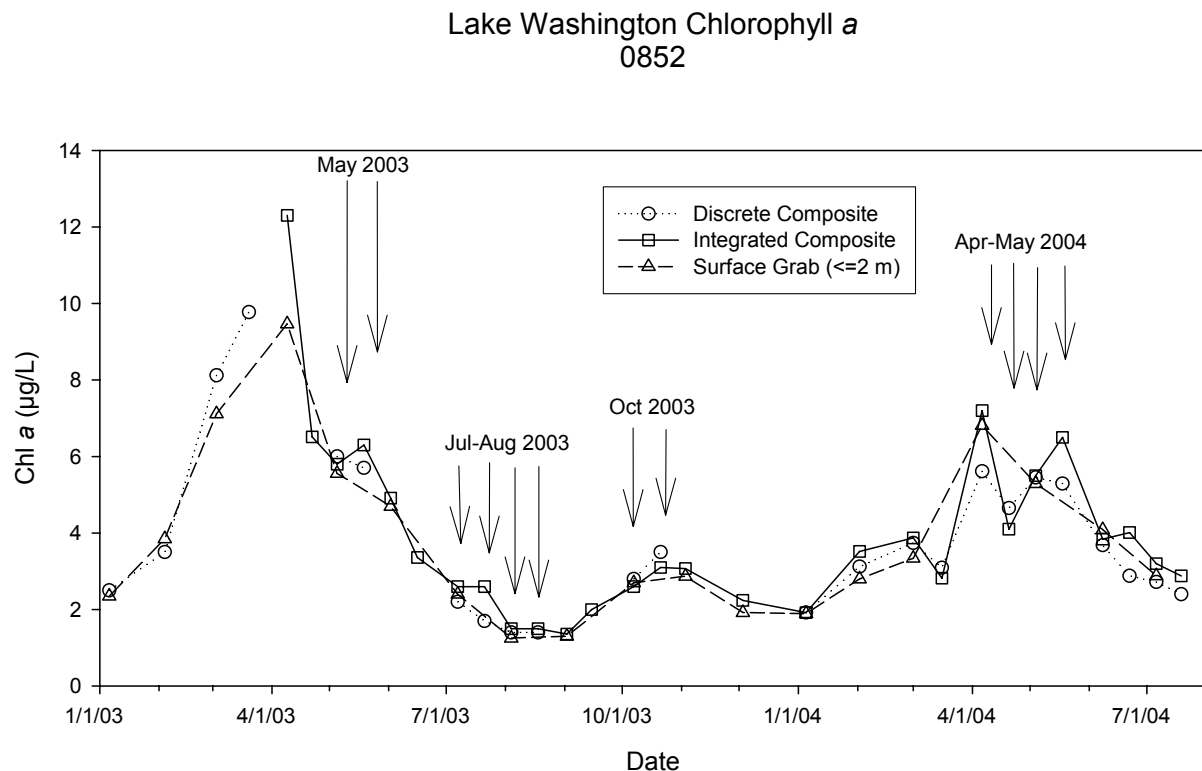


Figure 3 Chlorophyll *a* data for Lake Washington Station 0852 during the paired composite sampling study. Monthly surface grab data also shown for comparison.

3.1 Individual Paired Comparisons

The paired sampling results are summarized in Table 2 and Appendix Figures A1-A12. Statistically significant differences were detected between integrated vs. discrete composite chlorophyll *a* results in 6 of the 12 sampling events with significant differences ranging from

-0.4 to 1.2 µg/L. Note that a negative difference indicates that the integrated composite sample result was less than the discrete composite sample result.

Table 2. Results from the paired composite sampling for chlorophyll *a* in µg/L.

Date	n	Discrete D	± sd	Integrated I	± sd	Difference [I - D]	Significance <i>p</i> < 0.05	Secchi m
Spring 2003								
5/5/03	10	6.0	0.2	5.8	0.2	-0.2	0.011	3.8
5/19/03	10	5.7	0.1	6.3	0.3	0.6	5.0E-06	3.0
Summer 2003								
7/7/03	5	2.2	0.1	2.6	0.1	0.4	9.1E-04	4.5
7/21/03	5	1.7	0.2	2.6	0.2	0.9	5.6E-05	5.0
8/4/03	5	1.4	0.1	1.5	0.1	ns	0.055	7.8
8/18/03	5	1.4	0.1	1.5	0.1	ns	0.54	7.5
Fall 2003								
10/7/03	10	2.8	0.2	2.6	0.2	ns	0.085	5.3
10/21/03	10	3.5	0.1	3.1	0.1	-0.4	5.0E-06	7.0
Spring 2004								
4/6/04	5	5.9	0.5	7.2	0.3	1.2	0.0012	3.8
4/20/04	5	4.1	0.1	4.1	0.3	ns	-	4.5
5/4/04	5	5.2	0.2	5.5	0.2	ns	0.12	3.0
5/18/04	5	6.1	0.5	6.5	0.3	ns	0.11	3.0

ns = Difference between discrete and integrated composite sample not statistically significant.

3.2 Seasonal Comparisons

When the data are grouped into seasons (May 2003, Jul-Aug 2003, Oct 2003, Apr-May 2004), statistically significant differences were only detected in the Jul-Aug 2003 and Oct 2003 periods with differences of 0.4 and -0.2 µg/L, respectively (Table 3 and Appendix Figures A13-A16).

Table 3. Seasonally aggregated results from the paired composite sampling for chlorophyll *a* in µg/L.

Date	n	Discrete x	± sd	Integrated x	± sd	Difference [I - D]	Significance <i>p</i> < 0.05
Spring 2003	20	5.9	0.2	6.1	0.4	ns	0.051
Summer 2003	20	1.7	0.4	2.1	0.6	0.4	0.023
Fall 2003	20	3.1	0.4	2.9	0.3	-0.2	0.030
Spring 2004	20	5.3	0.9	5.8	1.2	ns	0.15

ns = Difference between discrete and composite sample not statistically significant.

4.0. DISCUSSION

Although the observed differences between the two methods were statistically significant in half of the comparisons on an individual and seasonal basis, it is questionable if the small differences observed (-0.4 to 1.2 µg/L) would significantly affect our ability to detect long term trends in seasonally averaged chlorophyll *a* concentrations in these lakes. A statistical analysis of the possible effect of these small differences on long term trend analyses is beyond the scope of this study. Instead of attempting to address this issue as part of this study, it was recommended at the beginning of 2004 that we re-establish the use of the discrete compositing technique for chlorophyll *a* at selected mid-lake locations (Lake Sammamish: 0611 and 0612; Lake Washington: 0826, 0852, and 0890; Lake Union: A522). This should allow us to continue the long-term collection of discrete composite chlorophyll *a* data for trend analysis.

Perhaps of more interest is an explanation for why the two sampling techniques frequently provided relatively small but significantly different results. The most logical explanation would be that there exists some consistent vertical structure in the chlorophyll *a* concentrations in the surface 10 m during a sampling event. Recall that one method integrates the concentrations over the surface 10 m and the other combines samples from 1 m and another depth that is typically less than 10 m. The Secchi depths measured during the paired comparison sampling study ranged from 3.0 to 7.8 m. Overall, Secchi depths at Station 0852 have ranged from 1.1 to 8.0 m between 1993 and 2003. Vertical structure in the chlorophyll *a* profile would potentially introduce some sampling bias between the two methods.

A look at the monthly discrete chlorophyll *a* profile data collected at Station 0852 in 2002, 2003, and 2004 suggests some vertical structure, especially during the spring diatom bloom (Figure 4). Direct comparison of the significantly different paired composite results to the available discrete profiles (May 5, 2003; July 7, 2003; April 6, 2004), indicates that the differences can be explained by the vertical structure in the chlorophyll *a* profiles (Figure 5). On May 5, 2003 surface chlorophyll *a* concentrations were higher than at 5 and 10 m depths. Since the Secchi depth was 3.8 m, the integrated composite result was lower than the discrete composite result by 0.2 µg/L. On July 7, 2003, the maximum chlorophyll *a* concentration was near the 10 m depth. Secchi depth was 4.5 m, so the integrated composite sample result was 0.4 µg/L higher than the discrete composite result. On April 6, 2004 the surface chlorophyll *a* concentration measured at 1 m was lower than the concentrations measured between 2 and 10 m depth. Since the Secchi depth was 3.8 m, the integrated composite sample was 1.2 µg/L higher than the discrete composite sample result.

In vivo chlorophyll *a* fluorescence profiles could potentially reveal additional information regarding the vertical structure of phytoplankton chlorophyll *a*, although fluorescence profiling has a number of limitations that include calibration, sensor sensitivity and scaling, temperature and photoinhibition effects, and sampling frequency and averaging issues (YSI Environmental no date). Since mid-2000, King County has deployed up to 3 Remote Underwater Sampling System (RUSS) profilers in Lake Washington and 2 in Lake Sammamish that conduct pH, temperature, dissolved oxygen, specific conductance, and chlorophyll fluorescence profiling up to 4 times per day at each station. However, the Yellow Springs Instruments (YSI) fluorometers used in this system have not been reliably accurate for the following reasons:

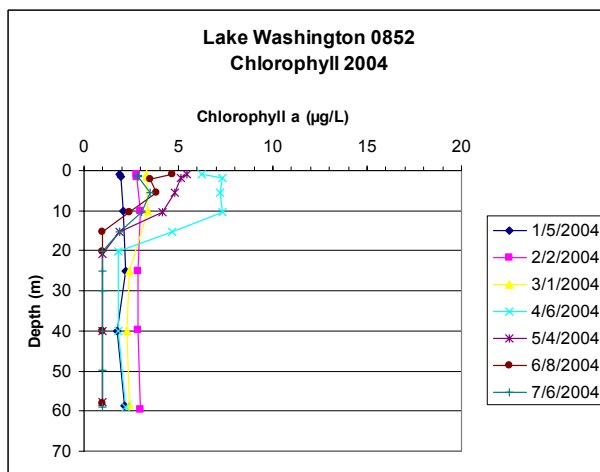
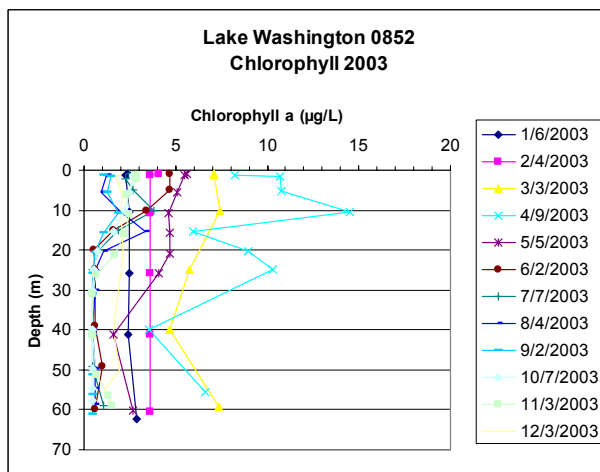
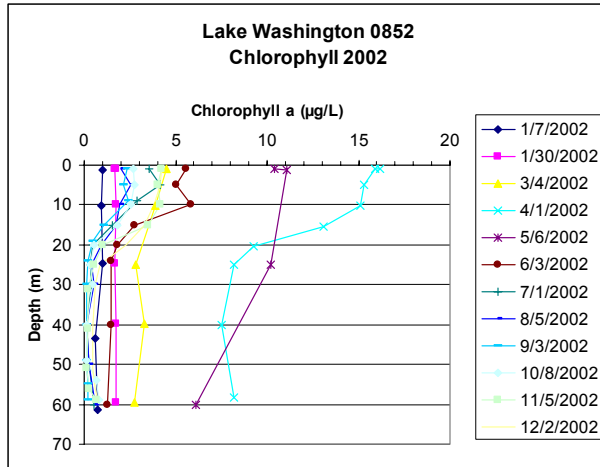


Figure 4 Discrete grab profiles of chlorophyll a for Station 0852 in 2002, 2003, and 2004.

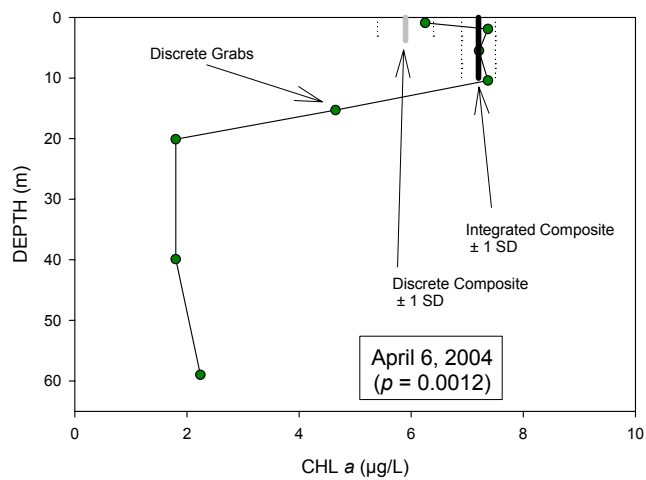
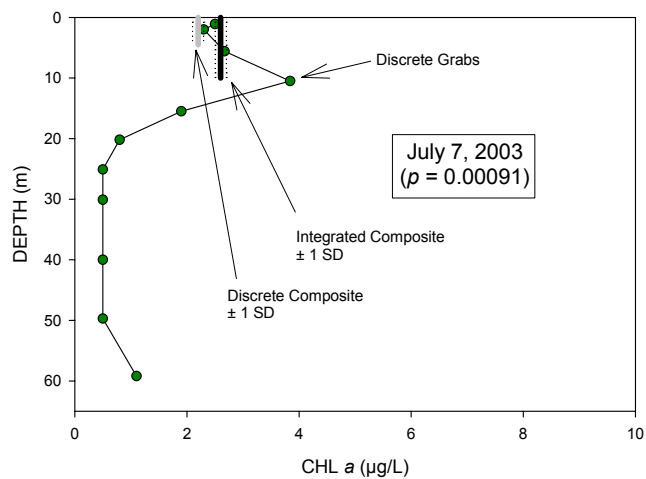
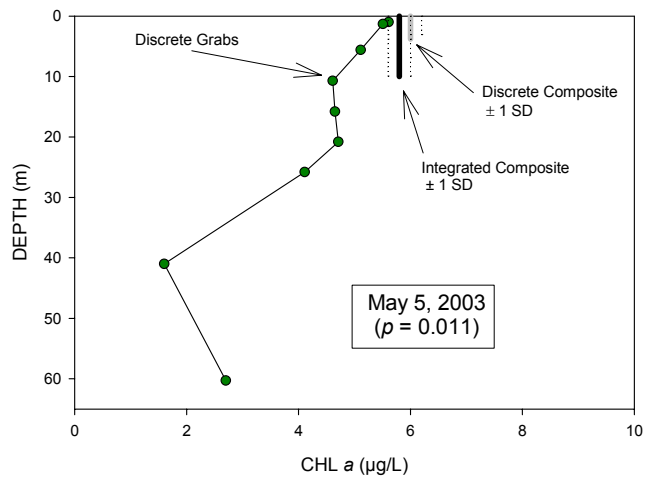


Figure 5 Comparison of discrete grab profiles of chlorophyll a for Station 0852 with significantly different discrete and integrated composite results.

- They have not been systematically calibrated to in-lake chlorophyll *a* measurements (for example see Figure 6)
- They do not sample frequently enough (or do not average enough frequent samples) during each profile resulting in non-representative sampling
- They have been out of commission for extended periods of time leaving gaps in the data set (see Figure 7)

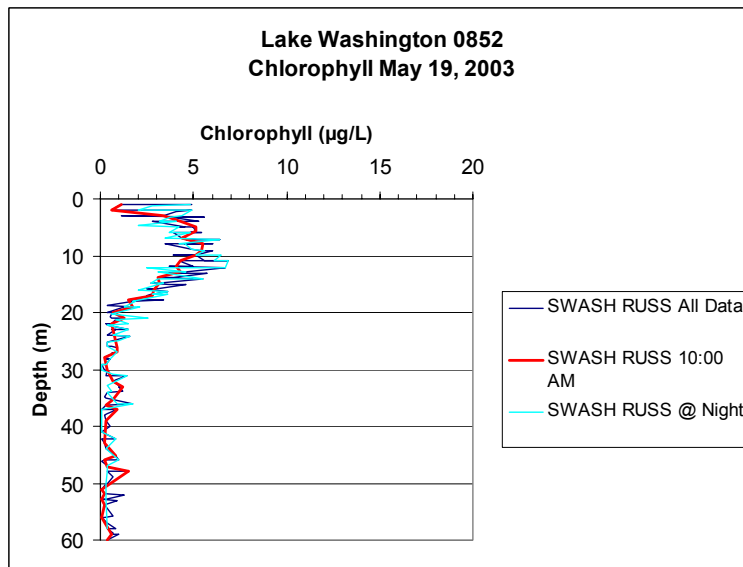
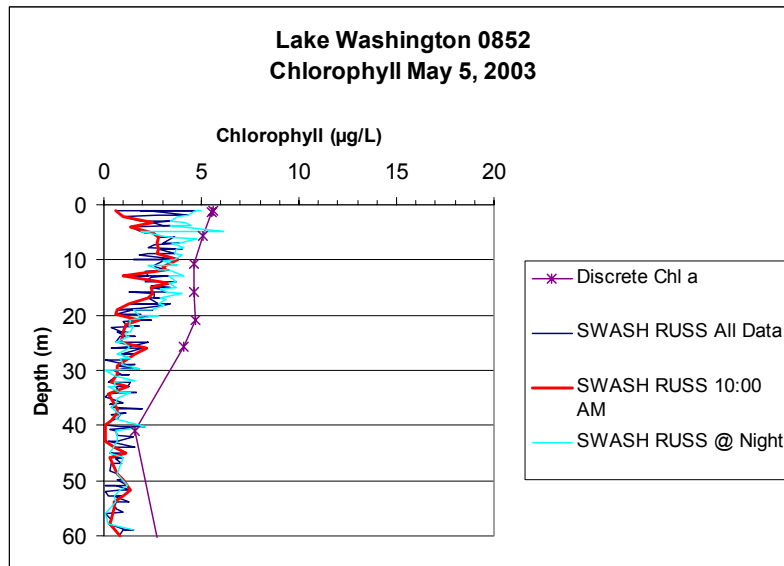


Figure 6 South Lake Washington (WASHS) RUSS chlorophyll profiles, May 5 and 19, 2003.

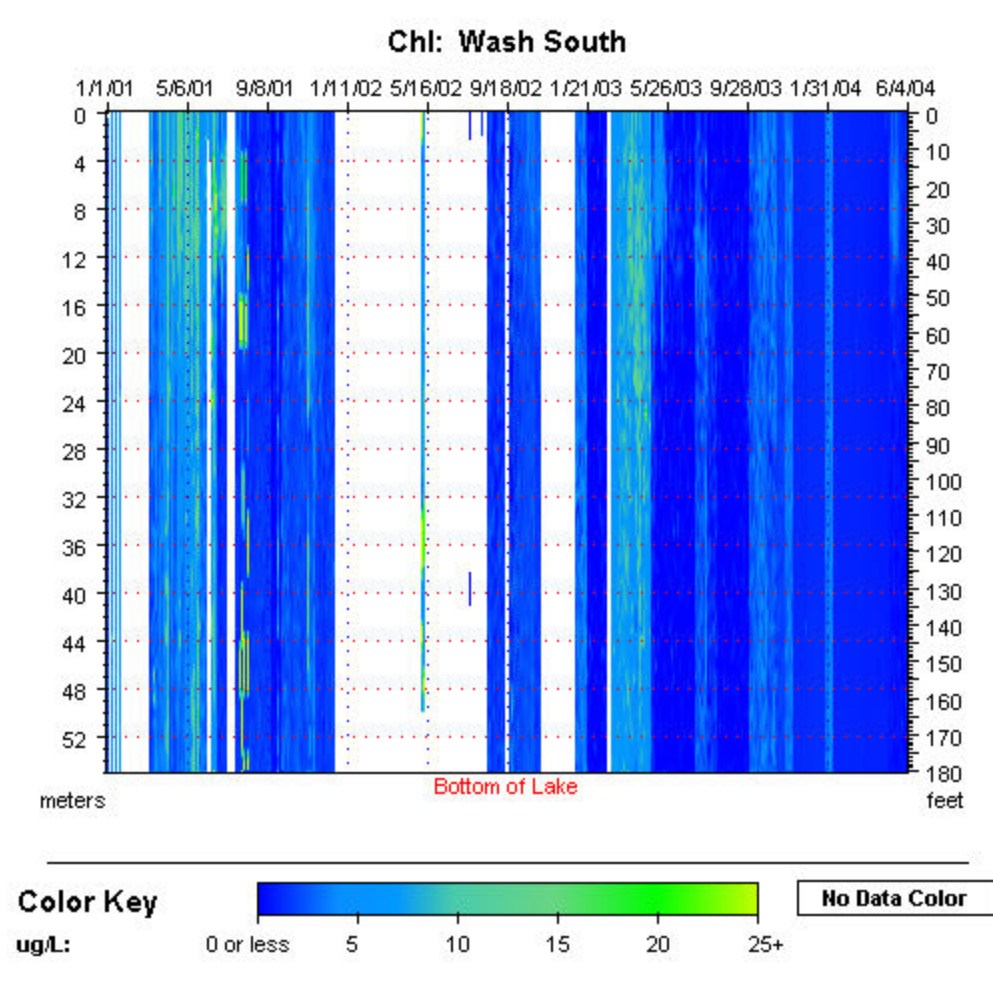


Figure 7 South Lake Washington (WASHS) RUSS chlorophyll color contour plot, 2001-2004.

Fluorescence profiling has also been conducted using a Self-Contained Autonomous Micro-Profiler (SCAMP) as part of a hydrodynamic study of Lake Washington and Lake Sammamish (King County 2002). High resolution temperature and fluorescence profiles have been collected anywhere from weekly to monthly at several stations in both lakes as part of the hydrodynamic study. Although this sampling device provides an appropriate profiling sampling frequency (100 Hz) for *in vivo* fluorescence, the data are of limited quantitative utility (results are reported as instrument voltage response) due to the lack of calibration to in-lake chlorophyll *a* measurements. Nonetheless, the instrument response appears to be fairly consistent over the course of the study and provides a reasonably good picture of the seasonal variation in the vertical chlorophyll structure in Lake Washington and Lake Sammamish (Figure 8). Based on the SCAMP fluorescence profiles, a subsurface maxima appears to be a common feature of phytoplankton vertical structure in both lakes. Of particular interest perhaps is the appearance of elevated fluorescence at the thermocline in both lakes during summer 2003 (see Figure 8).

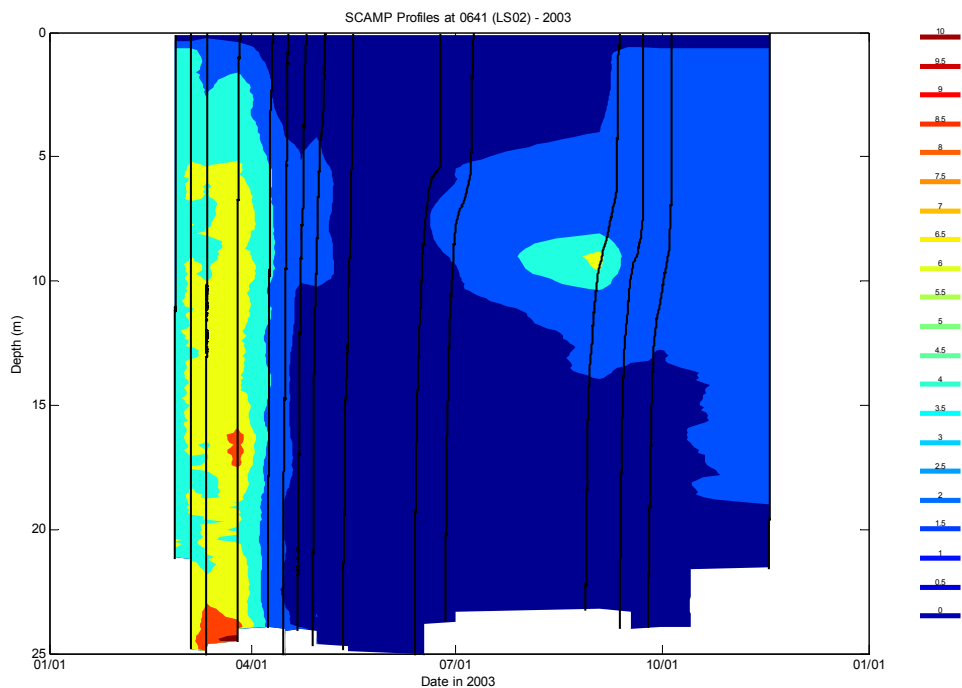
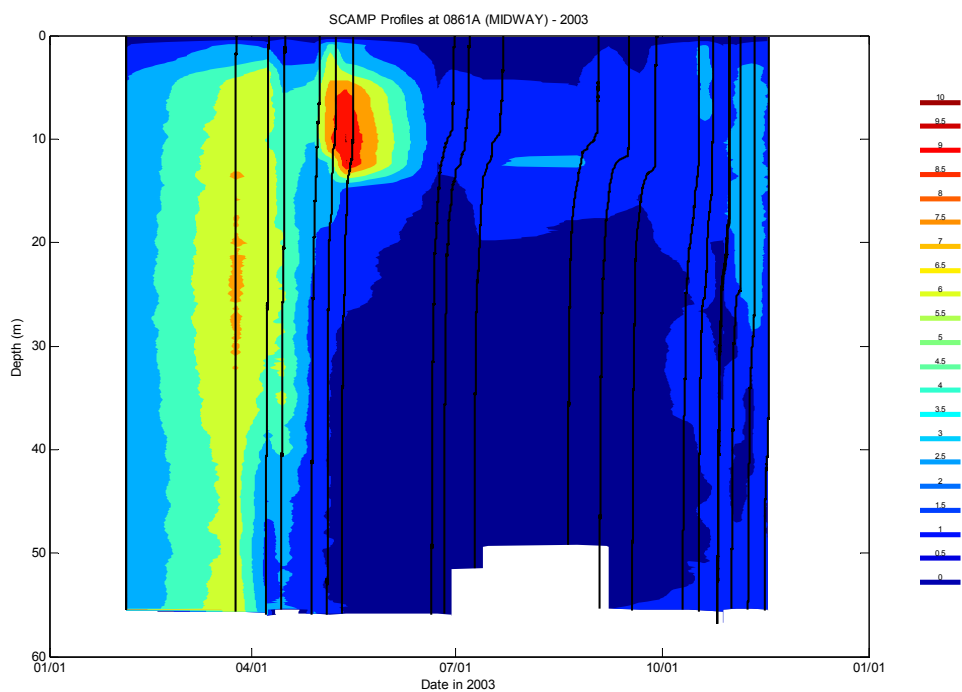


Figure 8 SCAMP fluorescence color contour plots (in units of voltage) for Lake Washington and Lake Sammamish, 2003. Black lines indicate date-centered temperature profiles associated with the interpolated fluorescence profiles.

Note that the minimum surface fluorescence observed during the spring diatom bloom should be interpreted with some caution. To some extent, the reduction of *in vivo* fluorescence at the surface is probably evidence of photoinhibition. Photoinhibition results in a reduction in *in vivo* fluorescence of phytoplankton exposed to relatively high light intensities, but does not necessarily indicate lower concentrations of chlorophyll or algal biomass (Heaney 1978). Heaney (1978) also noted that photoinhibition effects could be minimized with dark-adapted algae, that the *in vivo* fluorescence response of cyanobacteria was not as great as other phytoplankton, and that cyanobacteria did not exhibit photoinhibition. Therefore, the RUSS profiles collected at night during the spring diatom bloom might provide a more accurate picture of the vertical structure of the phytoplankton population during this period. Comparison of RUSS profiles collected during the day and at night on May 5th and 19th seem to support the photoinhibition hypothesis (see Figure 6). The vertical pattern of the nighttime RUSS profile is similar to the vertical discrete chlorophyll *a* concentrations measured on May 5th, while the daytime RUSS profile from that day indicates lower chlorophyll.

Discrete vertical chlorophyll *a* profiling is conducted only once a month at Station 0852. Therefore, only the South Lake Washington RUSS chlorophyll profiles are shown for May 19th in Figure 6. Even the nighttime *in vivo* fluorescence-derived chlorophyll profile indicates relatively lower chlorophyll content at the surface and an increase to a maximum concentration near the 10-m depth. Concentrations then decrease to levels similar to those measured at the surface by the 20 m depth. The fluorescence patterns measured using the SCAMP in late May corroborate the RUSS observations (Figure 8). This vertical pattern in estimated phytoplankton biomass would explain the significantly higher concentrations measured by the integrated compositing technique on May 19th since the discrete composite only represents the concentrations at the 1 and 3-m depth (see Table 2), while the integrated composite would include samples from the depth of maximum concentration. Photoinhibition does reduce the ability of phytoplankton to photosynthesize, so extended periods of photoinhibition would lead to lower phytoplankton biomass. Such an effect on the spring diatom bloom was hypothesized by Neale et al. (1991). Interestingly, Lehman et al. (2004) identified a negative relationship between net positive growth and an index for the mean light intensity in the surface mixed layer for many of the predominant diatom species in Lake Washington over the last 40+ years.

The vertical structure in the spring phytoplankton biomass was not expected. In preparation for this phytoplankton and composite sampling study, the 2002 hydrodynamic study fluorescence data were reviewed and data were only available beginning in May 2002. The May 30, 2002 Lake Washington profiles suggested some vertical structure, but the fluorometer went off scale and there were no other profiling dates that suggested that this pattern was typical. Review of the 2002 and 2003 discrete chlorophyll *a* profiles at Station 0852 (see Figure 4) suggest that the vertical structure of phytoplankton varies from season to season and year to year, but these profiles are too infrequent to identify any consistent patterns.

Using available routine monitoring discrete grab chlorophyll *a* profiles from Station 0852 and vertical profiles of fluorescence data from RUSS and SCAMP, it appears that the significant differences between the two compositing methods can be explained by the way the two methods sample the vertical distribution of phytoplankton. The integrated composite technique should

provide the best estimate of chlorophyll *a* integrated over the surface mixed layer (typically the surface 10 m during summer) and the discrete composite should provide a good estimate of the chlorophyll *a* content from the surface to the Secchi depth, which is typically less than the depth of the surface mixed layer. Due to these differences, and the concern that the utility of the data generated using the discrete composite technique for trend detection may be jeopardized if it were replaced by the integrated composite technique, the discrete composite sampling method has been re-established at a limited number of stations as part of the routine monitoring program.

5.0. CONCLUSIONS AND RECOMMENDATIONS

A number of conclusions can be made regarding the representativeness of the discrete vs. integrated compositing technique

- Integrated composite sampling is more representative of the concentrations in the surface mixed layer than the discrete compositing technique.
- The discrete compositing technique is more representative of the concentrations visible to surface observers. Therefore, this measurement technique is more relevant to assessing relationships with surface transparency and aesthetics.

Evaluation of the available discrete grab chlorophyll *a* profile data and chlorophyll fluorescence data from the RUSS and SCAMP instruments also highlights the importance of discrete vertical sampling to the overall understanding of long term trends and dynamics of phytoplankton biomass and species composition in our study lakes.

In a recent evaluations of 50 years (1950-1999) of phytoplankton data collected in Lake Washington as part of a long term University of Washington limnological study, a number of connections were made between chemical and physical variables and seasonal and long term changes in phytoplankton species composition and biomass (Lehman et al. 2004, Edmondson et al. 2003, Scheuerell et al. 2002). Of particular current interest is the increasing appearance in Lake Washington of *Microcystis*, a cyanobacterium capable of cyanotoxin production (Chorus et al. 2002). Edmondson et al. (2003) hypothesized that the progressive warming of Lake Washington during the last 50 years (Arhonditsis et al. 2004) may be leading to more physically stable conditions that provide a competitive advantage to *Microcystis*, which can regulate its buoyancy. The steady increase in lake alkalinity may also be playing a role in the rise in importance of *Microcystis* and other coccoid colonial cyanobacteria. In addition to these observed trends, changes in recent years in the relationships among available silica, nitrogen and phosphorus mediated by grazing of phytoplankton by *Daphnia* were hypothesized to lead to further changes in the composition of diatoms in Lake Washington (Edmondson et al. 2003).

I recommend that King County's Major Lakes sampling program coordinate closely with the University of Washington's studies of Lake Washington. More frequent discrete vertical sampling for chlorophyll *a* coupled with frequent **quantitative** fluorescence profiling are essential to improving our understanding of seasonal and inter-annual phytoplankton dynamics. Quantitative fluorescence profiling using the RUSS buoys will require some modification or replacement and routine calibration of the fluorometer to in-lake chlorophyll concentrations. Use of the SCAMP would require routine calibration, but the SCAMP has also been unreliable when used routinely in the field due to its sensitive electronics and it is also difficult to deploy under windy conditions.

Monitoring of phytoplankton, zooplankton, and fish species composition and biomass is currently being conducted in lakes Washington and Sammamish, but these programs could

probably be better integrated and it is unclear to what extent these monitoring programs will continue in the future (e.g., the King County phytoplankton sampling is currently planned to end in October 2004 and further zooplankton work on Lake Sammamish is uncertain). Re-establishment of phytoplankton productivity studies that were previously conducted in Lake Washington by the University of Washington should also be considered and should include Lake Sammamish as well. The limited total suspended solids, total organic carbon, and dissolved silica monitoring that has been conducted as part of SWAMP should also be incorporated into the design of King County's long term monitoring program.

Since a number of studies have shown that the spatial variability of nutrients, phytoplankton, and zooplankton is relatively low in Lake Washington and/or highly correlated spatially (Arhonditsis et al. 2003, Edmondson et al. 2003, Edmondson and Litt 1982), sampling more frequently at fewer locations and working more cooperatively with the University of Washington would provide the best hope of continuing to improve our understanding of how these lakes will respond to environmental change (e.g, population growth and climate change).

6.0. REFERENCES

- Arhonditsis, G.B., M.T. Brett, and J.D. Frodge. 2003. Environmental control and limnological impacts of a large recurrent spring bloom in Lake Washington, USA. *Environmental Management* 31: 603-618.
- Arhonditsis, G.B., M.T. Brett, C.L. DeGasperi and D.E. Schindler. 2004. Effects of climatic variability on the thermal properties of Lake Washington (USA). *Limnol. Oceanogr.* 49: 256-270.
- Chorus, I, I.R. Falconer, H.J. Salas, and J. Bartram. 2000. Health risks caused by freshwater cyanobacteria in recreational waters. *J. Toxicol. Environ. Health B. Crit. Rev.* 4:323-347.
- DeGasperi, C.L. 2003. Major lakes phytoplankton study – comparison of composite sampling techniques. Technical Memo to Jonathan Frodge, King County DNR&P, WLRD. June 18, 2003 (revised August 12, 2003).
- Edmondson, W.T. and A.H. Litt. 1982. *Daphnia* in Lake Washington. *Limnol. Oceanogr.* 27:272-293.
- Edmondson, W.T., S.E.B. Abella, and J.T. Lehman. 2003. Phytoplankton in Lake Washington: long-term changes 1950-1999. *Arch. Hydrobiol. Suppl.* 139:275-326.
- Heaney, S.I. 1978. Some observations on the use of the *in vivo* fluorescence technique to determine chlorophyll-*a* in natural populations and cultures of freshwater phytoplankton. *Freshwater Biol.* 8:115-126.
- King County. 2002. Sammamish-Washington Assessment and Modeling Program: Hydrodynamic Monitoring Program Sampling and Analysis Plan. King County, Department of Natural Resources and Parks (KCDNRP), Wastewater Treatment Division, Seattle, WA.
- King County. 2003. Sammamish-Washington Analysis and Modeling Program: Major Lakes Phytoplankton Sampling and Analysis Project Plan. King County, Department of Natural Resources and Parks (KCDNRP), Wastewater Treatment Division, Seattle, WA.

King County. 2004. Sammamish-Washington Analysis and Modeling Program: Major Lakes Phytoplankton 2004 Sampling and Analysis Project Plan. King County, Department of Natural Resources and Parks (KCDNRP), Wastewater Treatment Division, Seattle, WA.

Lehman, J.T., S.E.B. Abella, A.H. Litt, and W.T. Edmondson. 2004. Fingerprints of biocomplexity: Taxon-specific growth of phytoplankton in relation to environmental factors. *Limnol. Oceanogr.* 49:1446-1456.

Neale, P.J., S.I. Heaney, and G.H.M. Jaworski. 1991. Responses to high irradiance contribute to the decline of the spring diatom maximum. *Limnol. Oceanogr.* 36:761-768.

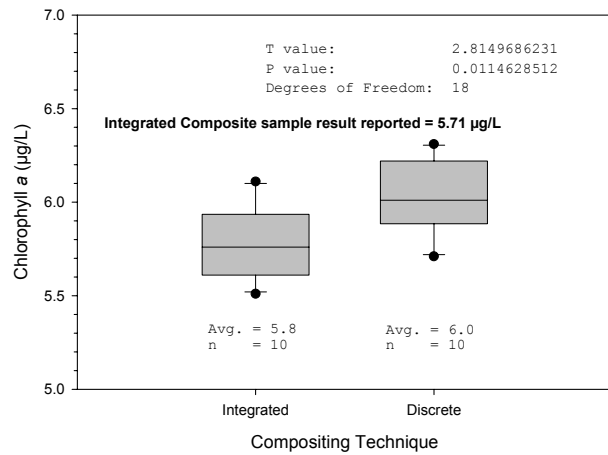
Scheuerell, M.D., D.E. Schindler, A.H. Litt, and W.T. Edmondson. 2002. Environmental and algal forcing of *Daphnia* production dynamics. *Limnol. Oceanogr.* 47:1477-1485.

YSI Environmental. No date. *In vivo* measurement of chlorophyll and the YSI 6025 wiped chlorophyll sensor. Yellow Springs Instruments (YSI) Environmental, Inc., Yellow Spring, OH.

Appendix A

Appendix Figures

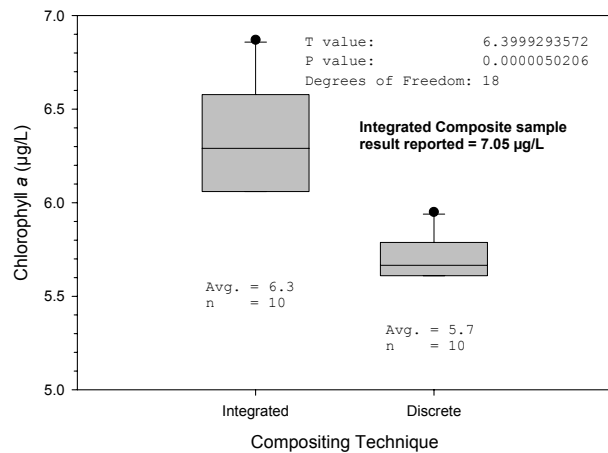
Lake Washington Chlorophyll a
Comparison of Compositing Techniques
May 5, 2003



Comparison of analytical results for chlorophyll a collected at Station 0852 on Lake Washington, May 5, 2003. Integrated composite was collected using a 10 m long tube to collect water from the surface to the 10 m depth. Discrete composite was collected by combining equal amounts of water collected from 1 m and the Secchi depth. The Secchi depth at this station was 3.8 m on this date.

Figure A1. Box plot comparing paired composite sampling results for May 5, 2003.

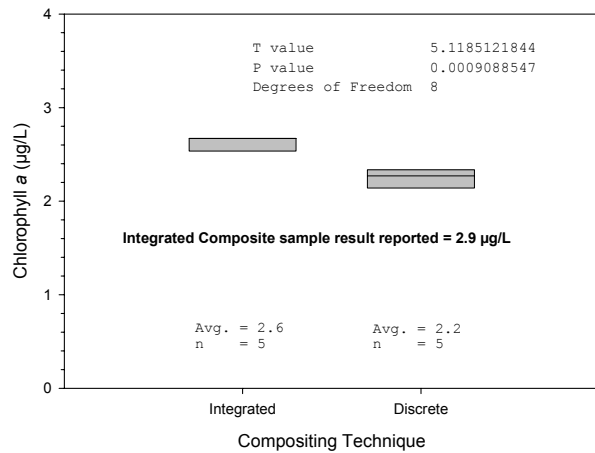
Lake Washington Chlorophyll a
Comparison of Compositing Techniques
May 19, 2003



Comparison of analytical results for chlorophyll a collected at Station 0852 on Lake Washington, May 19, 2003. Integrated composite was collected using a 10 m long tube to collect water from the surface to the 10 m depth. Discrete composite was collected by combining equal amounts of water collected from 1 m and the Secchi depth. The Secchi depth at this station was 3.0 m on this date.

Figure A2. Box plot comparing paired composite sampling results for May 19, 2003.

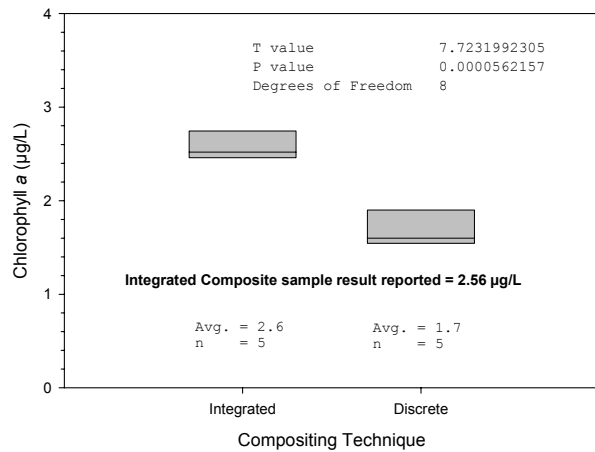
Lake Washington Chlorophyll a
Comparison of Compositing Techniques
July 7, 2003



Comparison of analytical results for chlorophyll a collected at Station 0852 on Lake Washington, July 7, 2003. Integrated composite was collected using a 10 m long tube to collect water from the surface to the 10 m depth. Discrete composite was collected by combining equal amounts of water collected from 1 m and the Secchi depth. The Secchi depth at this station was 4.5 m on this date.

Figure A3. Box plot comparing paired composite sampling results for July 7, 2003.

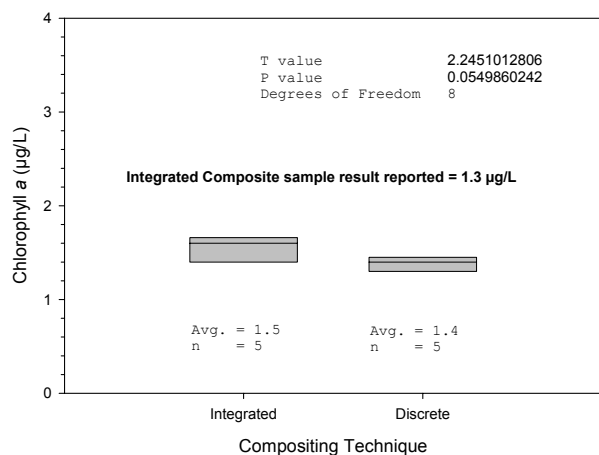
Lake Washington Chlorophyll a
Comparison of Compositing Techniques
July 21, 2003



Comparison of analytical results for chlorophyll a collected at Station 0852 on Lake Washington, July 21, 2003. Integrated composite was collected using a 10 m long tube to collect water from the surface to the 10 m depth. Discrete composite was collected by combining equal amounts of water collected from 1 m and the Secchi depth. The Secchi depth at this station was 5.0 m on this date.

Figure A4. Box plot comparing paired composite sampling results for July 21, 2003.

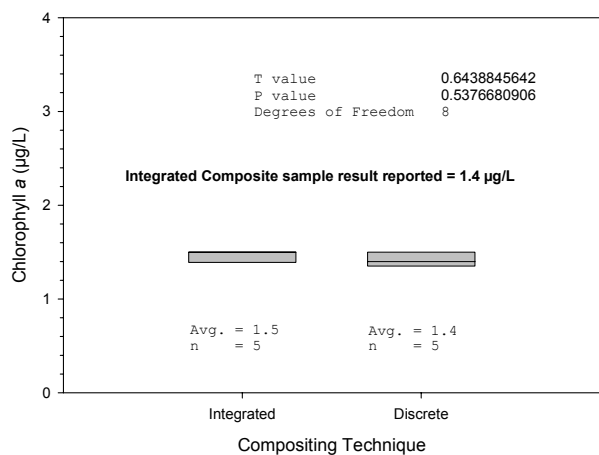
Lake Washington Chlorophyll a
Comparison of Compositing Techniques
August 4, 2003



Comparison of analytical results for chlorophyll a collected at Station 0852 on Lake Washington, August 4, 2003. Integrated composite was collected using a 10 m long tube to collect water from the surface to the 10 m depth. Discrete composite was collected by combining equal amounts of water collected from 1 m and the Secchi depth. The Secchi depth at this station was 7.8 m on this date.

Figure A5. Box plot comparing paired composite sampling results for August 4, 2003.

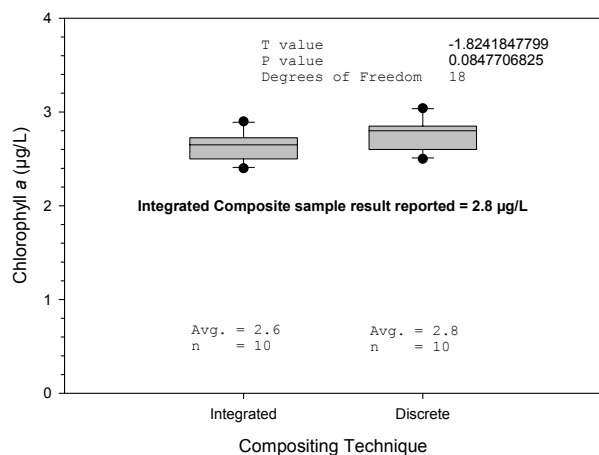
Lake Washington Chlorophyll a
Comparison of Compositing Techniques
August 18, 2003



Comparison of analytical results for chlorophyll a collected at Station 0852 on Lake Washington, August 18, 2003. Integrated composite was collected using a 10 m long tube to collect water from the surface to the 10 m depth. Discrete composite was collected by combining equal amounts of water collected from 1 m and the Secchi depth. The Secchi depth at this station was 7.5 m on this date.

Figure A6. Box plot comparing paired composite sampling results for August 18, 2003.

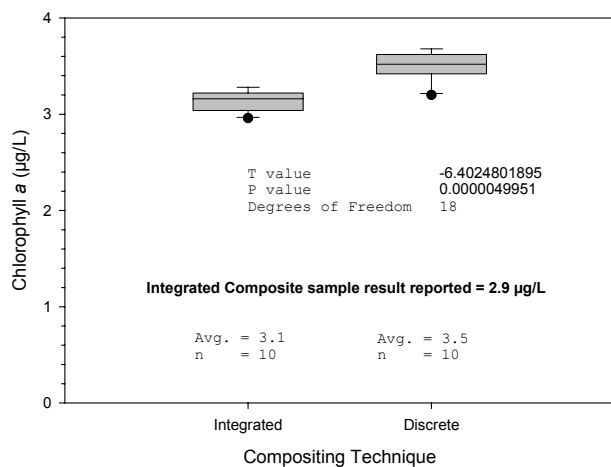
Lake Washington Chlorophyll a
Comparison of Compositing Techniques
October 7, 2003



Comparison of analytical results for chlorophyll a collected at Station 0852 on Lake Washington, August 7, 2003. Integrated composite was collected using a 10 m long tube to collect water from the surface to the 10 m depth. Discrete composite was collected by combining equal amounts of water collected from 1 m and the Secchi depth. The Secchi depth at this station was 5.3 m on this date.

Figure A7. Box plot comparing paired composite sampling results for October 7, 2003.

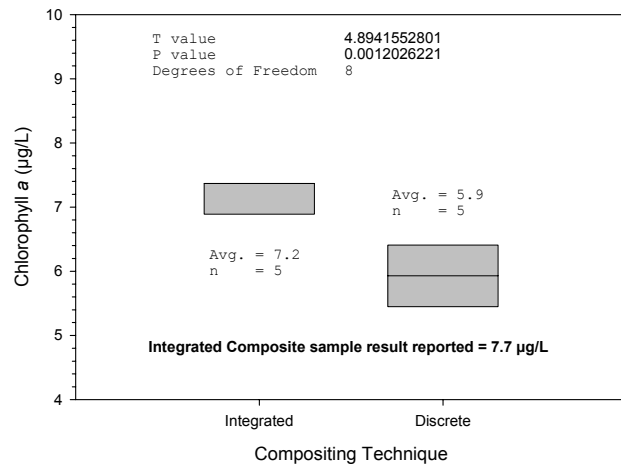
Lake Washington Chlorophyll a
Comparison of Compositing Techniques
October 21, 2003



Comparison of analytical results for chlorophyll a collected at Station 0852 on Lake Washington, August 21, 2003. Integrated composite was collected using a 10 m long tube to collect water from the surface to the 10 m depth. Discrete composite was collected by combining equal amounts of water collected from 1 m and the Secchi depth. The Secchi depth at this station was 7.0 m on this date.

Figure A8. Box plot comparing paired composite sampling results for October 21, 2003.

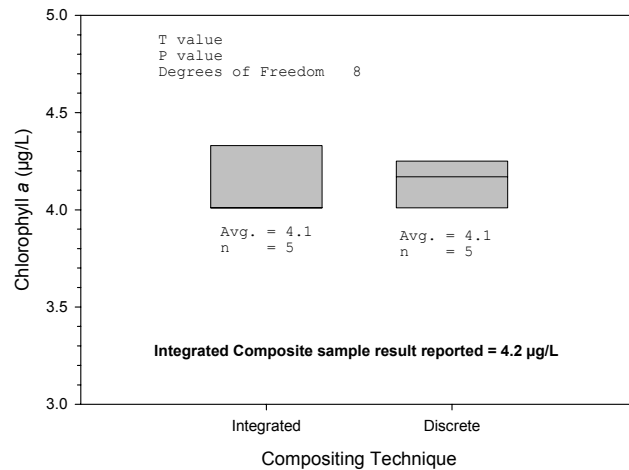
Lake Washington Chlorophyll *a*
Comparison of Compositing Techniques
April 6, 2004



Comparison of analytical results for chlorophyll *a* collected at Station 0852 on Lake Washington, April 6, 2004. Integrated composite was collected using a 10 m long tube to collect water from the surface to the 10 m depth. Discrete composite was collected by combining equal amounts of water collected from 1 m and the Secchi depth. The Secchi depth at this station was 3.8 m on this date.

Figure A9. Box plot comparing paired composite sampling results for April 6, 2004.

Lake Washington Chlorophyll *a*
Comparison of Compositing Techniques
April 20, 2004



Comparison of analytical results for chlorophyll *a* collected at Station 0852 on Lake Washington, April 20, 2004. Integrated composite was collected using a 10 m long tube to collect water from the surface to the 10 m depth. Discrete composite was collected by combining equal amounts of water collected from 1 m and the Secchi depth. The Secchi depth at this station was 4.5 m on this date.

Figure A10. Box plot comparing paired composite sampling results for April 20, 2004.

Lake Washington Chlorophyll *a*
Comparison of Compositing Techniques
May 4, 2004

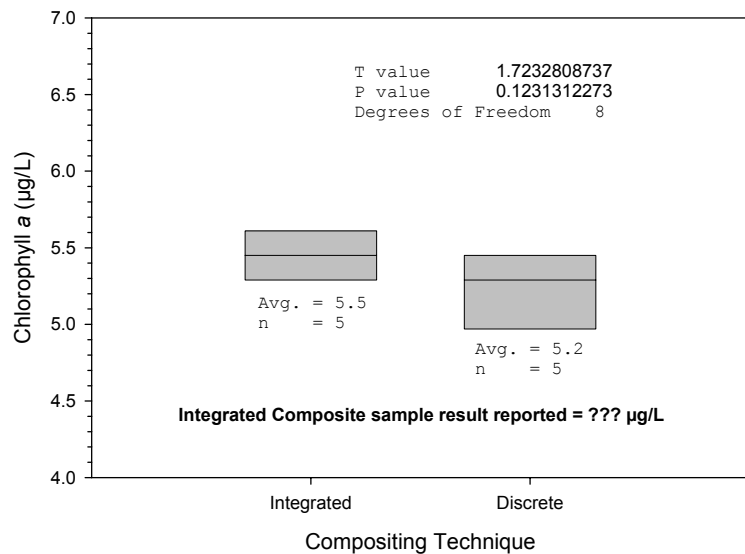


Figure A11. Box plot comparing paired composite sampling results for May 4, 2004.

Lake Washington Chlorophyll *a*
Comparison of Compositing Techniques
May 18, 2004

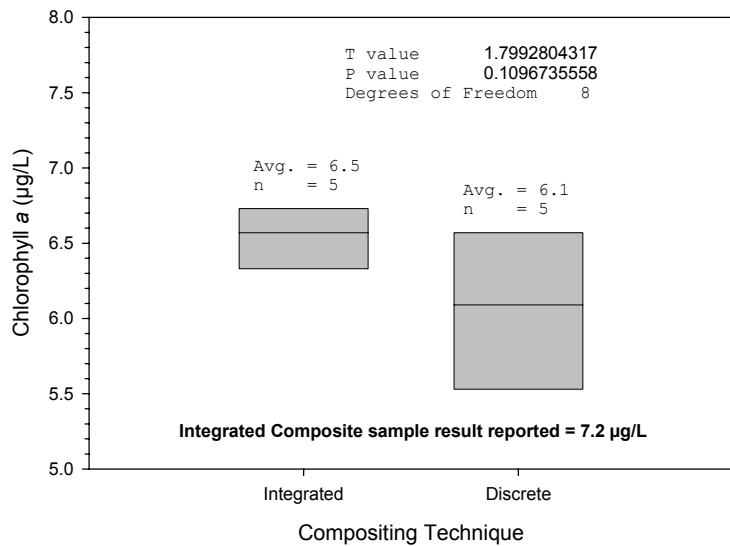
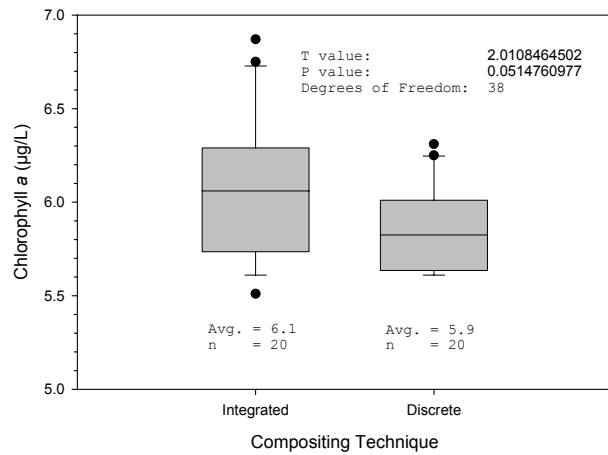


Figure A12. Box plot comparing paired composite sampling results for May 18, 2004.

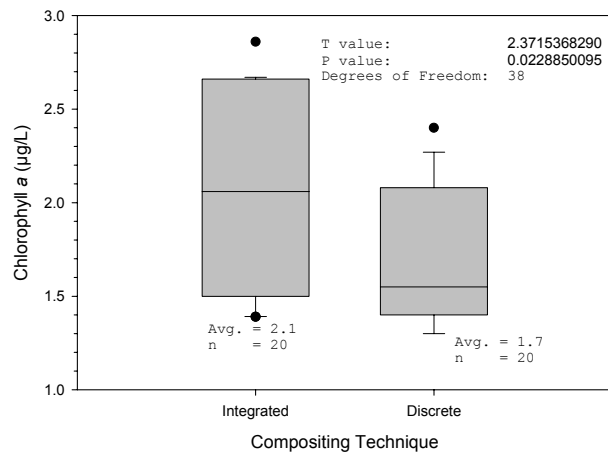
Lake Washington Chlorophyll a
Comparison of Compositing Techniques
Spring 2003 (May only)



Comparison of analytical results for chlorophyll a collected at Station 0852 on Lake Washington, Spring 2003. Integrated composite was collected using a 10 m long tube to collect water from the surface to the 10 m depth. Discrete composite was collected by combining equal amounts of water collected from 1 m and the Secchi depth.

Figure A13. Box plot comparing paired composite sampling results for Spring (May) 2003.

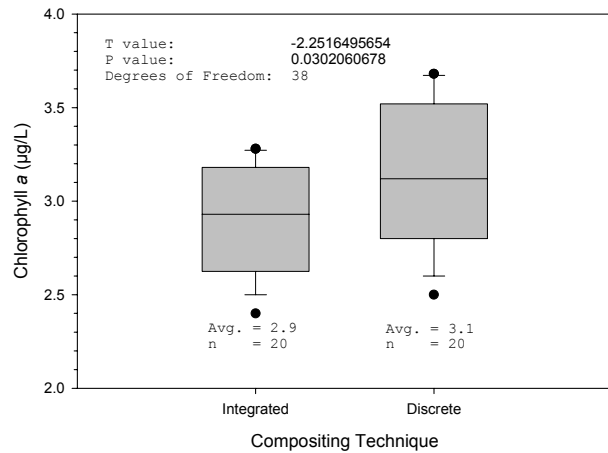
Lake Washington Chlorophyll a
Comparison of Compositing Techniques
Summer 2003 (July-August)



Comparison of analytical results for chlorophyll a collected at Station 0852 on Lake Washington, Summer 2003. Integrated composite was collected using a 10 m long tube to collect water from the surface to the 10 m depth. Discrete composite was collected by combining equal amounts of water collected from 1 m and the Secchi depth.

Figure A14. Box plot comparing paired composite sampling results for Summer (Jul-Aug) 2003.

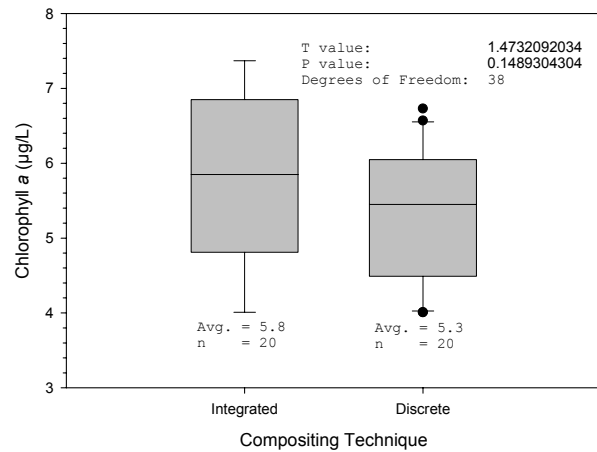
Lake Washington Chlorophyll a
Comparison of Compositing Techniques
Fall 2003 (October only)



Comparison of analytical results for chlorophyll a collected at Station 0852 on Lake Washington, Fall 2003.
Integrated composite was collected using a 10 m long tube to collect water from the surface to the 10 m depth.
Discrete composite was collected by combining equal amounts of water collected from 1 m and the Secchi depth.

Figure A15. Box plot comparing paired composite sampling results for Fall (Oct) 2003.

Lake Washington Chlorophyll a
Comparison of Compositing Techniques
Spring 2004 (April-May)



Comparison of analytical results for chlorophyll a collected at Station 0852 on Lake Washington, Spring 2004.
Integrated composite was collected using a 10 m long tube to collect water from the surface to the 10 m depth.
Discrete composite was collected by combining equal amounts of water collected from 1 m and the Secchi depth.

Figure A16. Box plot comparing paired composite sampling results for Spring (Apr-May) 2004.